A COMPARATIVE STUDY OF SEMINIFEROUS TUBULAR EPITHELIUM FROM RATS FLOWN ON COSMOS 1887 AND SL3

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ABSTRACT

Space flight, with its unique environmental constraints such as immobilization, decreased and increased pressures, and radiation, is known to affect testicular morphology and spermatogenesis. Among the several biological experiments and animals on board COSMOS 1887 Biosputnik flight were 10 rats, from which we have collected testicular tissue. Average weights of flight testes were 6.4% below that of the vivarium control when normalized for weight loss / 100 grams body weight. Counts of surviving spermatogonia per tubule cross section indicated an average of 39 spermatogonia for flight animals, 40 for synchronous controls and 44 for the vivarium controls. Serum testosterone was significantly decreased when compared to basal controls but the decrease was not significant when compared in vivarium and synchronous control groups. The significant decrease in spermatogonia and the decrease in serum testosterone are similar to that in animals flown on Space Lab 3 (Challenger Shuttle).

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Introduction

The testes have been shown to be affected by space flight, immobilization, irradiation and increased gravity. Fedorova (1) reported an increase of 30 to 70% atypical spermatozoa consisting of tail curling and absence of a tail. These abnormalities decreased to the high normal range of 30% by 75 days postflight. Rat testes from SL-3 showed a 7.5% decrease in Stage 6 spermatogonia and a weight loss of 7.1% when compared to controls (2). In earlier studies of rats flown aboard COSMOS 690 (3) and COSMOS 605 (4) no specific changes in the testes, directly attributed to flight, were reported. Immobilization, applied for a short or long period, is considered a form of physiological stress, and induces a decrease in plasma testosterone levels (5-7). While most reports indicate no change in the morphology of differentiating germ cells as a result of immobilization (8), a "striking" arrest of spermatogenesis in a primate has been reported (9). The sensitivity of the testes to radiation is well known and well studied. The details of spermatogonial effects of irradiation in mice, rats and primates have been the subject of studies utilizing X-irradiation and gamma irradiation (10-13). Alpen and Powers-Risius (14) have quantitated HZE irradiation effects using testes weight loss. Philpott et al.(15-19) have reported the results of cosmic (HZE) type irradiation on spermatogonial cell counts using an assay method that concentrates on cell numbers in spermatogonial Stage 6 (12) and which provides a means of detecting cell population changes at doses of less than 0.5 rads (17). This sensitive response makes the testes a possible candidate as a biological dosimeter.

Summary of Flight and Recovery

The following is from the "Summary of the COSMOS 1887 flight and specimen collection" as compiled by Dr. R.E. Grindeland, NASA:

The COSMOS 1887 Biosputnik flight was launched September 29, 1987 and landed October 12, 1987 after a flight of 12.5 days. Inflight, 10 male SPF rats of Czechoslovakian origin (Institute of Endocrinology, Brattislava, Czechoslovakia) were housed in a single cage which had 10 nozzles for delivery of a paste diet and 10 lixits for water. Fourteen-gram boluses of food (total 55g/day/rat) were provided ad libitum. The air pressure in the cage was 760 mm Hg, the humidity averaged 58%, and the ambient temperature was 22°C- 23°C. Lights were on from 0800-2400 and off from 2400-0800 hours. Light intensity was 4-8 lumens at the cage floor. The Biosputnik had an orbital inclination of 62.8 degrees; the apogee and perigee were 406 and 224 kilometers, respectively.

Due to difficulties during reentry, the Biosputnik landed in Siberia rather than the designated site where the recovery/ dissection team was located. The calculated landing force was less than 4 g. At the landing site there were about 15 cm of snow and the outside temperature varied from -5°C to -20°C. The Biosputnik was located in 3 hours and a rescue team placed a heated tent (23°C) around the vehicle. The following morning, 20 hours after landing, the animals were placed in transport cages and carried by bus (3 hours), airplane (6.5 hours), and van (0.5 hour) to the designated recovery/dissection site. Rats were sacrificed the next morning. Due to the recovery delays, rats had been without food for 42 hours. While the biosatellite was on the ground, the ventilation system continued to work so the animals

received an adequate air supply. The temperature of the rat cage was not recorded after landing, but calculations indicate that the temperature fell slowly and did not go below +12°C. When examined upon removal from the flight cage, the rats were somewhat dirty but apparently healthy and free from injury. During the airplane trip back from the landing site the animals "appeared exhausted" - much like the rats after the Space Lab 3 flight.

Methods

For each of the five animals in each group (flight, synchronous control and vivarium control) testes were removed, weighed, immediately slit open and immersed in cold Triple Fix (20). The specimens were kept at 4°C, shipped to Ames Research Center and refrigerated until time for processing. Samples were treated with 1% osmium tetroxide for 1 hour, dehydrated in ascending concentrations of acetone, infiltrated with Epon-Araldite and polymerized at 60°C for 48 hours. Six blocks were produced from each testis. Two-micron cross sections were cut on a Porter-Blum ultramicrotome and mounted on glass slides. The sections were stained with 1% toluidine blue in 1% borax. Alternate sections containing maturation Stage 6 (12) were scored for surviving spermatogonia.

Results

The average weight difference of the COSMOS flown rat testes is 6.4% as compared to vivarium controls when normalized for weight/100 grams. There is no difference in testis weight when flight animals and synchronous control animals are compared.

Counts of surviving spermatogonia (Table 1) per tubule cross section indicate an average of 38.79 spermatogonia for flight animals, 40.20 for synchronous controls and 43.75 for vivarium controls. The decreases of spermatogonia in flight tissues are significant when compared to synchronous control ($P<0.02$, 1 tail; $P<0.05$, 2 tail) and vivarium control ($P<0.0002$, 1 tail; $P<0.005$, 2 tail). Rats flown on SL-3 experienced a similar decrease in number of spermatogonia (2). Preliminary counts of Sertoli cells per tube cross section indicate no significant difference ($P>0.05$) when vivarium control animals are compared to either synchronous controls or to flight animals. This consistency in Sertoli cell numbers per tubule cross section demonstrates their stability under the adverse conditions of space flight and indicates that the minor volumetric changes in tubular epithelium due to spermatogonial cell loss do not affect the relative numbers per tubule of cross section. Spermatogonial cell loss can be quantitated per number of Sertoli cells or per tubule cross section. Changes in spermatogonial cell populations are indicative of actual cell loss and are not significantly influenced by volumetric changes in the tubules (Figure 1a, 1b, 1c).

Discussion

While it is general knowledge that the testes are very sensitive to certain environmental factors including stress and irradiation, not all reports agree on the extent and nature of morphologic changes in the seminiferous epithelia (8). Most reports indicate that stress decreases testosterone levels (5-7) but does not cause any morphological changes in seminiferous tubules (8). Interestingly, in human

beings, stress may cause either an increase or decrease in serum testosterone depending upon whether the stress is perceived as a threat to dominance/control (increased testosterone) or a loss of control (decreased testosterone) (7). On the other hand, irradiation, depending on the dosage, can result in the depletion of all spermatogonial cells except a few of the stem cells (17,13), but testosterone levels do not seem to be affected in the serum or intratesticular tissue by irradiation (21). Grindeland et al. (22) measured the serum testosterone in the COSMOS 1887 animals and reported lower hormone levels when flight animals were compared to vivarium and synchronous controls.

Previous space flight investigations prior to SL-3 have not reported changes in seminiferous epithelium while simulated conditions, at least in some investigators' labs, result in changes (4). Data obtained from rats flown on COSMOS 1887 indicated significantly reduced numbers of spermatogonia when compared to both synchronous control animals (4% decrease) and vivarium control animals (11% decrease) and were generally similar to results obtained from rats flown on SL-3 (2,23). Our assay procedure provided excellent quality specimens, sections thin enough to provide morphological differentiation of each spermatogonial cell class, and precise quantitation.

Data indicte a significant difference in spermatogonial population when the two control groups are compared. This difference may be caused by stresses encountered under simulated space flight conditions, or to as yet unexplained responses. A similar decrease in spermatogonia was seen in rats subjected to suspension in a simulated SL-3 flight (Table 1). We postulate that the decrease (4%) in spermatogonia observed in rats actually flown on COSMOS 1887 when compared to the synchronous controls is due to space flight conditions not adequately duplicated on the ground based synchronous experiment. One possible factor is radiation. Dosimetry reports from COSMOS 1887 indicate a dose factor of 0.313 rad at the dosimeter location within the space craft. Dosimeters were not located near the animals; therefore, the exact dosage received in that area is not known. Low radiation levels do not produce gross changes in morphology; however, spermatogonia near the first meiotic division are reduced in number. This is not unexpected and many investigations, including our own, substantiate these results (17). We have shown with X-rays and with HZE particles the extreme sensitivity and predictable response of the testicular epithelium to irradiation. The loss of cells not accounted for except by space travel could have resulted from radiation, especially since any particles penetrating the space craft would have been galactic and of similar energy to Iron. Our previous experiments indicate that irradiation with HZE particles of Iron at 0.5 rad level caused significant decreases in spermatogonia in mice (19) and these changes could be detected down to the 0.1 rad level (Unpublished).

Previous work in our laboratory led us to conclude that the testicular seminiferous epithelium is a good model for radiation sensitivity studies since it is composed of cellular populations which vary in individual radiosensitivities, indicated by the multiple slopes seen in spermatogonial survival curves (17). These characteristics of testicular epithelium provide an accurate means for biological dosimetric assessment of radiation exposure. Data collected from this flight (COSMOS 1887) as well as from the earlier SL-3 flight indicate that the biological effects of space flight are multifaceted. Impact on the morphophysiology of the testes through a number of different pathways, i.e., decreases in serum testosterone and testes weight loss, is observed in the animals described in this report. Stress related gonadal dysfunction and possible galactic radiation exposure, possibly along with other factors, apparently contribute to the significant decrease in spermatogonial cell numbers observed in rats flown in space. Various changes in the environment can alter testicular integrity. The site of action of these various environmental impacts and the mechanisms by which they interfere with both spermatogenesis and steroidogenesis need further investigation. These important investigations should be repeated with longer flights and a shorter time span between recovery and specimen preparation. When it becomes possible, fixation in flight will remove any doubt about the effect recovery may have.

Acknowledgments

This work was supported in part by the NASA/Ames Research Center Cooperative Agreement #NCC2-12 and #NC2-455 (to W.J.S.). Ultrastructural Facility support was provided by NIH/DRR/RCMI grant #G12RR03059-01A1.

Note: The work presented here also appears as a publication in the FASEB Journal.

References

1. Fedorova, N.L., Kosmichesda Biol Med., **1967**, 1, 28. Space Biol. Med. 1, 28.

2. Philpott, D.E.; Sapp, W.; Williams, C.; Fast, T.; Stevenson, J.; Black, S., Proc. EMSA., **1986**, 44, 248.

3. Plakhuta-Plakutina, G.I., Aviat. Space Environ. Med., **1977**, 48, 12.

4. Portugalov, V.V.; Savina, E.A.; Kaplansky, A.S.; Yakovleva, V.I.; Plakhuta-Plakutina, G.I.; Pankova, A.S.; Katunyan, P.I.; Shubich, M.G.; Buvalio, S.A., Aviat Space Environ. Med., **1976**, 47, 813.

5. Carpenet, G.; Tache, Y.; Forest, M.G.; Haour, G.; Saez, J.M.; Bernier, M.; Ducharme, J.R.; Collu, R., Endocrinology, **1981**, 109, 1254.

6. Armario, A.; Castellanos, J.M., Physiol. Behav., **1984**, 32, 517.

7. Collu, R.; Gibb, W.; Ducharme, J.R., J. Endocrinol. Invest., **1984**, 7, 529.

8. McGrady, A.V.; Chakraborty, J., Arch. Androl., **1983**, 10, 95.

9. Zemjanis, R.; Gondos, B.; Adey, W.R.; Crockett, A.T.K., Fertil. Steril., **1970**, 21, 335.

10. Oakberg, E.F.; Clark, E., J. Cell. Comp. Physiol., **1961**, 58 (Suppl 1), 173.

11. Hugon, J.; Borgers, V.M., Anat. Rec., **1966**, 155, 15.

12. Huckins, C., Anat. Rec., **1978**, 190, 905.

13. VanAlphen, M.M.A.; VanDeKant, H.J.G.; DeRooij, D.G., Radiat. Res., **1988**, 113,473.

14. Alpen, E.L.; Powers-Risius, P., Radiat. Res., **1981**, 88, 132.

15. Philpott, D.E.; Sapp, W.; Williams, C.; Stevenson, J.; Black, S., Proc. EMSA., **1981**, 39, 542.

16. Philpott, D.E.; Sapp, W.; Williams, C.; Stevenson, J.; Corbett, R.; Black, S., Proc. EMSA., **1983**, 41, 674.

17. Philpott, D.E.; Sapp, W.; Williams, C.; Stevenson, J.; Corbett, R.; Black, S., Scanning Electron Microsc., **1983**, III, 1345.

18. Philpott, D.E.; Sapp, W.; Williams, C.; Stevenson, J.; Black, S.; Corbett, R., The Physiologist., **1985**, 28(6)suppl, s211.

19. Philpott, D.E.; Sapp, W.; Williams, C.; Stevenson, J.; Black, S.; Corbett, R., Proc. EMSA., **1985**, 43, 652.

20. Philpott, D.E., Corbett, R.; Turnbill, C.; Black, S.; Dayhoff, D.; McGourty, J.; Lee, R.; Harrison, G.; Sovick, L.. Aviat. Space Environ. Med., **1980**, 51, 556.

21. Cunningham, G.R.; Huckins, C., Radiat. Res., **1978**, 76, 331.

22. Grindeland, R.; Popova, I.; Vasquez, M., FASEB J., **1989**, Submitted for publication.

23. Sapp, W.J.; Philpott, D.E.; Williams, C.S.; Kato, K.; Stevenson, J.; Serova, L.V., Proc. EMSA., **1988**, 46, 276.

TABLE 1. TOTAL SURVIVING SPERMATOGONIA PER STAGE 6 SEMINIFEROUS TUBULE PROFILE[a]

Treatment Group Data	Individual Animals[b]	Mean + S.E.M.[c]	SL-3[d]	SL-3[e] Simulation
Flight	37.90 ± 0.15 38.77 ± 0.12 39.36 ± 0.15 39.08 ± 0.12 38.83 ± 0.13	38.79 ± 0.06**	39.75 ± 0.14	26.82 ± 0.27
Synchronous	40.44 ± 0.10 41.13 ± 0.15 39.08 ± 0.19 40.00 ± 0.12 40.35 ± 0.12	40.20 ± 0.06*	N/A	N/A
Vivarium	43.15 ± 0.17 44.36 ± 0.13 43.58 ± 0.16 43.55 ± 0.20 44.12 ± 0.17	43.75 ± 0.07	42.71 ± 0.17	44.24 ± 0.27

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a Tubules identified according to Huckins (Anat. Rec. 190:905, 1978). Cross section only.

b 200 tubules scored per animal; mean ± standard error.

c 5 animals per treatment; total = 1000 tubules scored.

d SL-3 data shown here for comparison (See Philpott et al., EMSA 44:248, 1986). Mean ± standard error, 200 tubules scored.

e Rat tail suspension; 50 tubules scored; mean ± standard error

* Significantly different from vivarium control, $P<0.001$

** Significantly different from vivarium control, $P<0.0005$

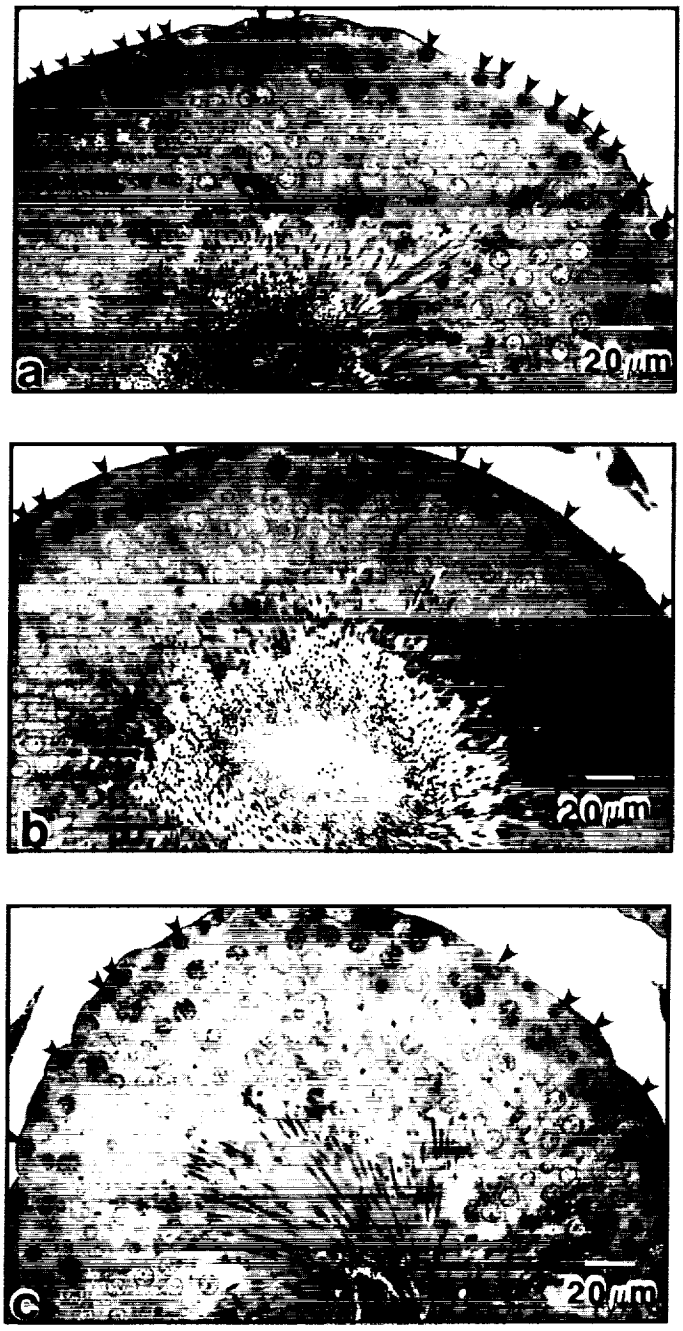

Figure legend

Figure 1. Representative sections of seminiferous tubules: (a) Vivarium control; (b) Synchronous control; (c) Flight animals. Arrows indicate spermatogonia.

Magnification = 300x.